**Aβ ELISA Protocol**

Aβ can be detected in tissue culture supernatant and in homogenized tissue. Two anti-Aβ antibodies will be needed, one for coating onto the ELISA plate for capture and a secondary biotinylated antibody for detection.

1. Coat ELISA 96-well plate with 100 μl per well of antibody (at a final concentration of 3.5 μg/mL) diluted in Well-Coating Buffer. Well-coating buffer may need to be warmed at 37ºC to bring it into solution. -Per plate (96 wells), make 10 mL antibody in Well-Coating Buffer.

2. Cover with plate sealer (tape interface to prevent evaporation) and incubate O/N at RT.

3. The next morning, aspirate Well-Coating Buffer.

4. Add 200 μl per well Well Fixing Buffer.

5. Seal and incubate ≥1 hr at RT.

6. Aspirate Well Fixing Buffer.

7. Add 100 μl/well standards, controls and unknowns. Use synthetic Aβ (either 1-40 or 1-42) standards, diluting serially from 1,000 pg/mL to 2 pg/mL in each ELISA plate.

8. Seal and incubate ≥1hr at RT.


10. Wash 3 x 1 min with TBS wash buffer.

11. Add 100 μl/well biotinylated secondary antibody (the optimal antibody concentration should be determined empirically after biotinylation) diluted in Specimen Diluent.

12. Seal and incubate 1hr at RT.


14. Wash 3 x 1 min with TBS wash buffer.

15. Add 100 μl/well HRP-Avidin D diluted 1:2000 in Specimen Diluent.

16. Seal and incubate 1 hr at RT (begin warming substrate).
17. Aspirate supernate.

18. Wash 3 x 1 min with TBS wash buffer.

19. Add 100 µl/well QuantaBlu (warmed to RT), mixed 9 parts Substrate Solution with 1 part Stable Peroxide Solution.

20. Seal and incubate 1 hr at RT. Check the reaction development with UV light, if desired.

21. Add 100 µl/well Stop Solution and mix by gently shaking the plate.

22. Read on a fluorometer, (excitation 340, emission 400).

**Reagents**

*ELISA Plate*
- FluoroNunc MaxiSorp, high binding
- Flat bottom, no lid.

*HRP-Avidin D*
- Vector Labs A-2004 5mg/ml

*QuantaBlu*
- Pierce #15169

**Well Coating Buffer (1 L)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium phosphate monobasic (monohydrate)</td>
<td>0.23g</td>
</tr>
<tr>
<td>Sodium phosphate dibasic</td>
<td>26.2g</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>1.0g</td>
</tr>
<tr>
<td>MilliQ H2O</td>
<td>1 L</td>
</tr>
</tbody>
</table>

*Caution:* sodium azide releases poisonous gas at acidic pH
1. Dissolve phosphates in 950 mL H2O, stir well.
2. Adjust pH to between 8.3 and 8.7.
3. Add sodium azide.
4. Add water to final volume.
5. Stir well and filter through 0.2 µm membrane.
6. Store 4°C.

**0.25% Well Fixing Buffer**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose, crystalline</td>
<td>25g</td>
</tr>
<tr>
<td>Sodium phosphate dibasic</td>
<td>10.8g</td>
</tr>
<tr>
<td>Sodium phosphate monobasic</td>
<td>1.0g</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>0.5g</td>
</tr>
<tr>
<td>HSA, 30% solution (Sigma A-6909)</td>
<td>8.33 mL (BSA may substitute)</td>
</tr>
<tr>
<td>MilliQ H2O</td>
<td>992 mL</td>
</tr>
</tbody>
</table>
1. Dissolve phosphates and sucrose in 950 mL H20.
2. Adjust pH to between 7.2 and 7.6.
3. Add sodium azide.
4. Add HAS and stir until dissolved.
5. Add water to final volume.
6. Stir well and filter through 0.2 μm membrane.
7. Store 4°C.

**Specimen Diluent**

Sodium phosphate monobasic 0.2g
Sodium phosphate dibasic 2.16g
Thimerosal 0.5g
Sodium chloride 8.5g
Triton x-405 0.5mL
BSA, globulin free (Sigma A-7030) 6.0g
MilliQ Water 1L

*Caution:* Thimerosal is poisonous.

1. Dissolve phosphates, Thimerosal, and NaCl in 950 mL H20.
2. Add triton X-405, rinsing pipette well.
3. Add BSA and stir well.
4. Adjust pH to between 7.3 and 7.5.
5. Add water to final volume, stir well.
6. Sterile filter through 0.2 μm membrane.
7. Store 4°C.

**10X TBS, pH 7.5 at 1x**

Sodium chloride 80.0g
Potassium chloride 3.8g
Tris base 5.85g
Tris HCl 31.75g
MilliQ H20 1 L

1. Dissolve salts in 950 mL H20.
2. Make a 1X dilution with 1 mL of 10x in 9 mL H20. pH should be between 7.5 and 7.8. Adjust 10X solution until diluted TBS is correct.
3. Sterile filter through 0.2 μm membrane.
4. Store 4°C.

**TBS Wash Buffer** - TBS-T 0.05% Tween 20, pH 7.5
20X TBS (pH 7.5 at 1X)  50 mL
Tween 20    0.5 mL
MilliQ H20    949.5 mL

1. Combine ingredients and mix well.

Antibodies should be tested for their compatibility with the ELISA assay. For this protocol, the following antibodies have been used successfully:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Epitope</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>2G3</td>
<td>Abeta 40 C-terminus</td>
<td>Elan Pharmaceuticals</td>
</tr>
<tr>
<td>21F12</td>
<td>Abeta 42 C-terminus</td>
<td>Elan Pharmaceuticals</td>
</tr>
<tr>
<td>266</td>
<td>Abeta residues 13-28</td>
<td>Elan Pharmaceuticals</td>
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<tr>
<td>3D6</td>
<td>Abeta 1-5 N-terminus</td>
<td>Elan Pharmaceuticals</td>
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